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16. ABSTRACT Pattern reversal evoked potentials (PREPs), flash evoked potentials (FEPs), optic nerve axonal transport, and body temperature were measured in hooded rats treated with either saline or the formamidine insecticide/acaricide, chlordimeform (CDM). Rats receiving CDM had low body temperatures when housed at standard laboratory room temperature, 22°C, but not at 30°C. FEP peak latencies were prolonged by CDM at 22°C, but not at 30°C. The rate of axonal transport was slowed in CDM-treated hypothermic rats, but not in CDM-treated warmed rats. These findings suggest that the FEP and optic nerve axonal transport changes produced by CDM were a secondary consequence of hypothermia. In contrast, CDM produced increased PREP peak latencies and peak-to-peak amplitudes which were independent of body temperature. These findings confirm and extend previous reports of CDM-induced hypothermia, emphasize the importance of body temperature changes as a possible confounding factor in studies of neuroactive agents, and demonstrate that CDM has both body-temperature dependent and independent actions in the rat visual system.		
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BODY TEMPERATURE-DEPENDENT AND INDEPENDENT ACTIONS OF CHLORDIMEFORM ON VISUAL EVOKED POTENTIALS AND AXONAL TRANSPORT IN OPTIC SYSTEM OF RAT

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Summary—Pattern-reversal-evoked potentials (PREPs), flash-evoked potentials (FEPs), rapid axonal transport in the optic system and body temperature were measured in hooded rats, treated with either saline or the formamidine insecticide/acaricide, chlordimeform (CDM). Rats receiving chlordimeform had low body temperatures when housed at standard laboratory room temperature, 22 C, but not at 30 C. Peak latencies of flash-evoked potentials were prolonged by chlordimeform at 22 C, but not at 30 C. The rate of axonal transport was slowed in chlordimeform-treated hypothermic rats, but not in chlordimeform-treated warmed rats. These findings suggest that the flash-evoked potential and axonal transport changes produced by chlordimeform were an indirect consequence of hypothermia. In contrast, chlordimeform increased pattern-reversal evoked potential peak latencies and peak-to-peak amplitudes independent of body temperature. These findings confirm and extend previous reports of chlordimeform-induced hypothermia, emphasize the importance of changes in body temperature as a possible confounding factor in studies of neuroactive agents and demonstrate that chlordimeform has both body-temperature-dependent and independent actions in the visual system in the rat.

Key words: chlordimeform, formamidine pesticide, pattern-reversal-evoked potentials, flash-evoked potentials, visual-evoked potentials, axonal transport, hypothermia.

Apart from a number of behavioral studies (e.g. MacPhail and Leander, 1981), little is known concerning the actions of chlordimeform (CDM), a formamidine insecticide/acaricide, on the intact mammalian nervous system. It has been found that acute exposure to chlordimeform caused changes in evoked potentials recorded from the visual and auditory systems of the rat. The amplitude of components in pattern-reversal-evoked potentials (PREPs), but not flash-evoked potentials (FEPs), was increased by chlordimeform, yet the latency of both responses was prolonged (Boyes and Dyer, 1984). In auditory-evoked potentials recorded in the brainstem, chlordimeform changed the amplitude of only component N4-P4, but increased the latency of all peaks occurring after the initial peak, P1 (Janssen, Boyes and Dyer, 1983). The selective nature of changes in amplitude of evoked potentials induced by chlordimeform, in contrast to the general nature of the changes in latency, suggested that the evoked potentials were disrupted by chlordimeform through at least two processes.

Among the manipulations which can disrupt evoked potentials are changes in neurotransmitter systems (e.g. Dyer, Howell and MacPhail, 1981), body temperature (e.g. Dyer and Boyes, 1983) and axonal transport (Holmgren, Karlsson and Sjostrand, 1978; Perisic and Cuenod, 1972). The variety of actions of chlordimeform, recently reviewed (Hol-

lingworth and Lund, 1982; Knowles, 1982; Matsumura and Beeman, 1982), includes inhibition of monoamine oxidase and possibly hypothermia (Pfister and Yim, 1977). The present authors have recently shown that changes in evoked potentials induced by chlordimeform were not an indirect consequence of inhibition of monoamine oxidase (Boyes, Moser, MacPhail and Dyer, 1985; Janssen *et al.*, 1983). The peak latencies of evoked potentials increase following decreases in body temperature (e.g. Dyer and Boyes, 1983). The increase of latencies of evoked potentials produced by chlordimeform lasted between 6 and 24 hr after treatment (Boyes and Dyer, 1985), a comparable time course to the reduced body temperature produced by chlordimeform in rats pretreated with pyrogen (Yim, Holsapple, Pfister and Hollingworth, 1978). Unfortunately, in the latter study, the body temperatures of rats receiving chlordimeform without pretreatment with pyrogen were not reported. These findings raise the possibility that the increased peak latencies of visual-evoked potentials in rats treated with chlordimeform were an indirect consequence of hypothermia, rather than a direct effect of chlordimeform on the visual system.

Apparently, no measurements of axonal transport after treatment with chlordimeform have been reported. Treatment with colchicine, a potent inhibitor of rapid axonal transport, produced significant changes in the visual-evoked potentials of rabbits

(Holmgren *et al.*, 1978) and pigeons (Perisic and Cuenod, 1972). In addition, axonal transport is highly dependent on temperature (e.g. Gross, 1973). Because rapid axonal transport may be required for the maintenance of normal synaptic function, the possibility was investigated that the effects of chlordimeform on visual-evoked potentials were the result of decreased rapid axonal transport was investigated.

Three sets of experiments were conducted to determine whether hypothermia and/or disruption of axonal transport could account for any of the alterations in visual-evoked potentials induced by chlordimeform. The first sought to confirm the effect of chlordimeform on body temperature. The second investigated whether the prevention of hypothermia could eliminate changes in visual-evoked potentials induced by chlordimeform. The third examined the effect of chlordimeform on rapid axonal transport of radiolabelled glycoproteins in retinal ganglion cells, and the dependence of this effect on body temperature.

METHODS

Animals

Adult male Long-Evans hooded rats (Charles River), weighing 250–400 g, were used in all experiments. The rats were individually housed in plastic cages with wood chip bedding. The lighting cycle was 12:12 light:dark with lights on at 6:00 a.m. Drinking water and food (Purina Lab Chow, 5001) were continuously available.

Body temperature

To determine the magnitude and time course of changes in body temperature produced by chlordimeform, rats ($n = 4/\text{group}$) were treated intraperitoneally (i.p.) with either the isotonic saline vehicle (1 ml/kg.) or 40 mg/kg of chlordimeform HCl (NOR-AM Agricultural Products, Naperville, Illinois; total salt). Immediately after injection, each rat was placed in a small plastic restraining tube, a thermal probe (YSI No. 402) was inserted approx. 10 cm into the rectum and taped in place and rectal temperature was measured using a YSI 44TA Tele-thermometer (Yellow Springs, Ohio) every 30 min for 6.5 hr. Room temperature was maintained at about 23.5°C.

Electrophysiology

For evoked potential studies, the general experimental procedures were similar to those described elsewhere (Boyes and Dyer, 1984). Briefly, chronic epidural stainless-steel electrodes were surgically implanted (chloropent anesthesia), at least one week prior to experimentation. For each rat, the active recording electrode was placed over the visual cortex, 6 mm posterior to the bregma and 4 mm lateral to the midline. Reference and ground electrodes were placed

2 mm anterior to the bregma and 2 mm to the right and left of the midline, respectively.

Rats were harness-restrained for measuring pattern-reversal-evoked potentials, flash-evoked potentials and rectal temperatures. The stimulus eliciting pattern-reversal-evoked potentials was a black and white square-wave grating (99% contrast, 30 cd/m², 69 × 74° field size), generated on the T.V. screen of a Grass Model VPG pattern generator. The stimulus eliciting flash-evoked potentials was the strobe flash of a Grass model PS-22 photostimulator, set at an intensity of 16, which provided a 10-μsec flash of approx. 4.53×10^7 lx peak power. Responses were evoked at a rate of 0.5 Hz. Both pattern-reversal-evoked potentials and flash-evoked potentials were averaged over 200 trials. Rectal temperature was recorded during each evoked potential recording session. Body temperature was manipulated by setting the laboratory temperature at either standard room temperature (23°C), or at 30°C.

The rats ($N = 32$) were randomly assigned to one of four treatment groups that received either saline or 40 mg/kg of chlordimeform (i.p.) 1 hr before testing and were tested at a room temperature of either 22 or 30°C. Pattern-reversal-evoked potentials and flash-evoked potentials were recorded from all rats in a counterbalanced order. The statistical analysis of the data followed the guidelines of Muller, Otto and Benignus (1983).

Axonal transport

Ninety minutes after an injection of chlordimeform or saline, the animals were injected (under brief ether anesthesia) intraocularly (posterior vitreous chamber) with 50 μCi (1 μl) of [³H]fucose (New England Nuclear, Corp. Boston, Massachusetts, L-[6-³H]-fucose, 10–15 Ci/mmol). The rectal temperature of the animals was monitored with a Yellow Springs model 44TA Tele-thermometer. Two axonal transport experiments were conducted with identical protocols except that in the first the rats were housed at standard room temperature and in the second the rectal temperature of the treated animals was maintained within the normal range by placing the animals in plastic restraining tubes on heating pads.

Animals were sacrificed by decapitation 3 hr after injection of the isotope and several components of the visual system were quickly removed and frozen at –70°C. The dissected areas consisted of the ipsi- and contralateral optic nerves, optic tracts, superior colliculi and retinas. Acid-soluble and acid-precipitable radioactivity were separated by homogenizing each tissue piece in 10% trichloroacetic acid (TCA)–0.5% phosphotungstic acid solution and the precipitates were washed in succession with cold 5% trichloroacetic acid, ethanol and ether (Padilla and Morell, 1980).

The amount of acid-soluble label in each sample of tissue was determined by counting an aliquot of the supernatant in Scintiverse (Fisher Scientific, Pitts-

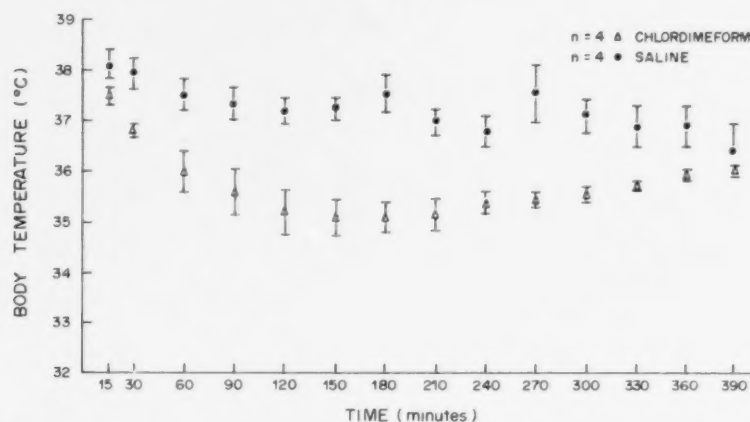


Fig. 1. Rectal temperatures of rats treated with either saline or 40 mg/kg chlordimeform (i.p.) and held in plastic restraining tubes. Room temperature was about 23.5°C. In this and subsequent Figures, the values plotted represent group means $\pm$ SEM. The body temperature of the rats was reduced by chlordimeform at standard room temperature.

burg, Pennsylvania). The acid precipitable radioactivity was determined by digesting the dried precipitate with 0.5 ml Protosol (New England Nuclear Corp., Boston, Massachusetts) containing 10% water, followed by addition of 10 ml of PPO-xylene scintillation cocktail (3 g 2,5-diphenyl oxazole in 1000 ml xylene). All scintillation counting was done on a Beckman LS 7000 liquid scintillation counter with a counting efficiency of 25%. Corrections for quenching were made if the external standard showed any significant variation.

RESULTS

The time course of changes in rectal temperature in rats receiving saline or 40 mg/kg chlordimeform is plotted in Fig. 1. The mean body temperature of control rats was initially above 38°C, probably elevated as a result of stress associated with restraint. Control body temperatures declined during the initial 60 min and remained between 36.5 and 38°C throughout the next 5 hr. The rats treated with chlordimeform showed a more rapid loss of body temperature during the first 2 hr after treatment, reaching a minimum rectal temperature of approx. 35°C. From about 120 until 210 min, the temperature was fairly stable, after which time the group treated with chlordimeform gradually regained body temperature. By 390 min after treatment, the body temperature of the rats treated with chlordimeform was about 36°C and the mean was within the control group standard error of the mean.

The mean rectal temperature of the rats during recording of evoked potentials is plotted in Fig. 2. The rats treated with chlordimeform exhibited a 2°C decrease in rectal temperature at a room temperature of 22°C. These body temperature values were similar

to those of the first experiment (Fig. 1) at the equivalent treatment time of 1 hr. When the room temperature was increased from 22 to 30°C, the mean rectal temperature of both groups increased. Temperatures of control groups rose from about 38 to about 39°C, and temperatures of the group treated with chlordimeform from about 36 to about 38.5°C. The rectal temperature of rats treated with chlordimeform and housed at 30°C was not significantly different from that of control rats housed at either 22 or 30°C.

The influence of room temperature and treatment with chlordimeform on the peak latencies of evoked

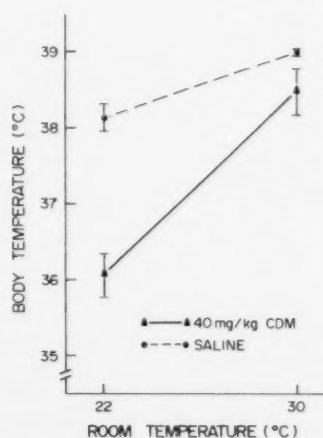


Fig. 2. Rectal temperatures of rats treated with saline or chlordimeform (CDM) during recording sessions of evoked potentials at two different room temperatures, 1 hr after injection. The rats were restrained in a harness for recording evoked potentials. Rats treated with chlordimeform were hypothermic at 22°C but not at 30°C.

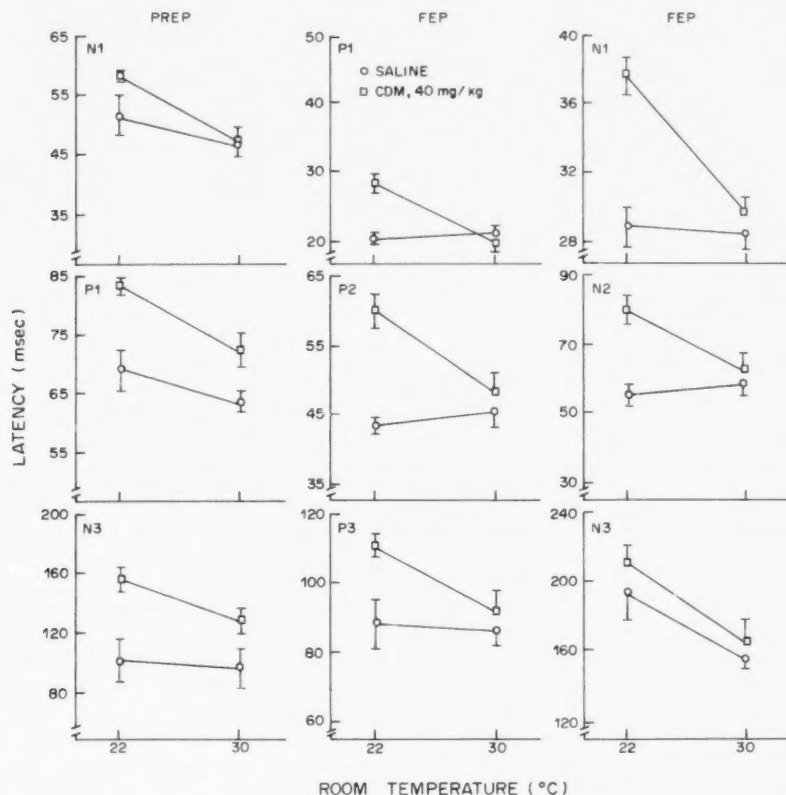


Fig. 3. Peak latencies of visual-evoked potentials as a function of room temperature and treatment with either saline or chlordimeform (CDM). Although the increases in peak latency of pattern-reversal-evoked potentials (PREP) produced by chlordimeform appeared to be less at 30 than 22°C, there was no statistically-significant interaction between dose and room temperature. The increases in peak latency of flash-evoked potentials (FEP) produced at 22°C were entirely eliminated at 30°C, suggesting that the increased latencies of flash-evoked potentials were an indirect consequence of hypothermia.

potentials is shown in Fig. 3. The latencies of peak P1 and N3 of the pattern-reversal-evoked potentials were longer in the group treated with chlordimeform than in controls at both room temperatures. A significant dose-effect was found in all the measured latencies of the pattern-reversal-evoked potentials [MANOVA $F(3,26) = 8.31$, $P = 0.0005$] and in analysis of variance for peaks P1 and N3 [both $F's(1,28) > 18.42$, both $P's < 0.0002$]. The magnitudes of the increases in latency induced by chlordimeform were similar to those previously reported (Boyes and Dyer, 1984). By increasing room temperature to 30°C, the latency of pattern-reversal-evoked potential peaks was reduced. The peak latency showed a significant effect of room temperature [MANOVA $F(3,26) = 4.48$, $P = 0.0116$] and significant differences for peaks N1 and P1 [both $F's(1,28) > 8.34$, both $P's < 0.0074$] but not for N3 [$F(1,28) = 2.69$, $P = 0.1124$]. There was no significant interaction between dose and room temperature for peak latencies of pattern-reversal-evoked potentials

[MANOVA $F(3,26) = 1.03$, $P = 0.3945$]. Thus, for peaks P1 and N3, the effects of chlordimeform on latency were independent of temperature.

The values of peak latency of the flash-evoked potential are also presented in Fig. 3. Similar effects were observed for peaks P1, N1, P2, N2, and P3. Treatment with chlordimeform prolonged the peak latency of flash-evoked potentials at 22°C but not at 30°C. Statistical analysis revealed a significant effect of dose [MANOVA $F(6,28) = 8.01$, $P < 0.0001$], a significant effect of room temperature [MANOVA $F(6,23) = 5.99$, $P = 0.0007$] and a significant interaction between dose and room temperature [MANOVA $F(6,23) = 4.10$, $P = 0.0061$]. Breakdown analysis of variance showed significant effects of dose and interactions between dose and room temperature for peaks P1, N1, P2, and N2 [all $F's(1,28) > 9.70$, all $P's < 0.0042$]. Increasing room temperature decreased the latency of N3 of both groups [ANOVA $F(1,28) = 12.43$, $P = 0.0015$] independent of treatment with chlordimeform. Room temperature, then,

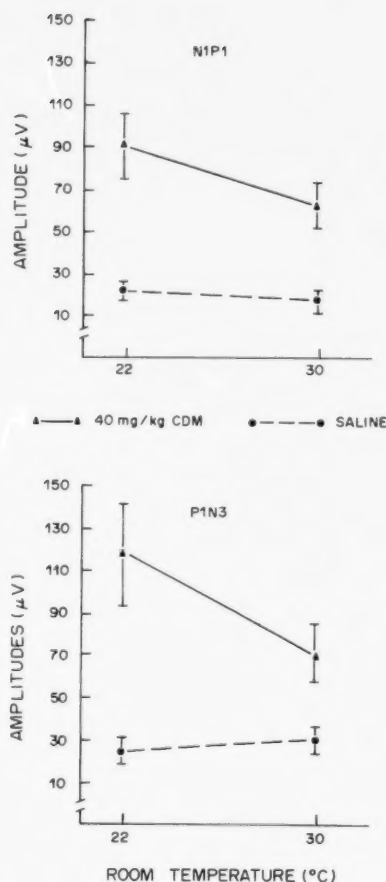


Fig. 4. Peak-to-peak amplitudes of pattern-reversal-evoked potentials as a function of both room temperature and treatment with either saline or chlordimeform (CDM). Although somewhat diminished, the amplitudes of pattern-reversal-evoked potentials in the group treated with chlordimeform were still twice the control values at 30°C.

had a strong influence on changes in latency of flash-evoked potential induced by chlordimeform. Latencies were increased at 22°C, but not at 30°C.

The peak-to-peak amplitudes of pattern-reversal-evoked potentials in rats treated with chlordimeform or saline are shown as a function of room temperature in Fig. 4. Amplitudes of pattern-reversal-evoked potentials were increased by chlordimeform at both 22 and 30°C. Although the magnitude of the effect of chlordimeform appeared to be smaller at the warmer room temperature, the peak amplitudes were still 2–3 times greater than those of the saline-treated group. Statistical analysis of peak-to-peak amplitudes of pattern-reversal-evoked potentials yielded a significant effect of dose [MANOVA $F(2,27) = 19.86$, $P < 0.001$], but no significant effect of room temperature or interaction between dose and room temperature [both MANOVA F 's(2,27) < 2.18 ,

both P 's > 0.1321]. The amplitudes of both N1P1 and P1N3 were increased by chlordimeform [both ANOVA F 's(1,28) > 24.32 , both P 's < 0.0001]. Raising room temperature to 30°C did not abolish the effect of chlordimeform on the amplitudes of pattern-reversal-evoked potentials.

In the studies of axonal transport, the level of acid-precipitable radioactivity in the non-injected retinal ganglion cells (i.e. contralateral retina and nerve and ipsilateral tract and superior colliculus) was less than 5% of the injected side. This indicates that the labelling of the injected retinofugal projections occurred via axonal transport rather than through the circulation.

Treatment with chlordimeform did not impair the incorporation of fucose into macromolecules in the retina. During the 3 hr between intraocular injection and sacrifice, $27 \pm 5\%$ (mean $\pm$ SE) of the injected radioactivity was incorporated into macromolecules in the rats treated with chlordimeform, as compared to $30 \pm 5\%$ in the saline-treated controls. Even though the synthesis of glycoprotein was comparable, axonal transport of these glycoproteins was not. Treatment with chlordimeform decreased the amount of [^{3} H]fucose-labelled glycoproteins in the contralateral optic tract and superior colliculus to 67 and 56% of control, respectively, as shown in Fig. 5A. However, when the body temperature of the rats treated with chlordimeform was kept within the control range, the amount of [^{3} H]fucose-labelled glycoproteins rapidly transported to the optic tract and superior colliculus was equivalent to control (Fig. 5B).

DISCUSSION

The changes in visual-evoked potentials and axonal-transport produced by chlordimeform were strongly dependent upon body temperature, but not all the changes induced by chlordimeform were secondary to hypothermia. At normal room temperatures, the body temperatures of rats treated with 40 mg/kg of chlordimeform decreased to a maximum of 2–2.5°C below that of controls. Under these conditions, the peak latencies of flash-evoked potentials and pattern-reversal-evoked potentials were prolonged and the rate of axonal transport slowed in rats treated with chlordimeform. When the body temperature of rats treated with chlordimeform was maintained within control range either by raising the room temperature to 30°C or by placing the animals on heating pads, flash-evoked potential latencies and rapid axonal transport parameters were not different from controls. For pattern-reversal-evoked potentials, prevention of hypothermia diminished the increases in peak latency included by chlordimeform, but the latencies of peaks P1 and N3 remained greater than controls. This suggests that the changes in flash-evoked potentials and axonal transport were an indirect consequence of hypothermia, but that chlor-

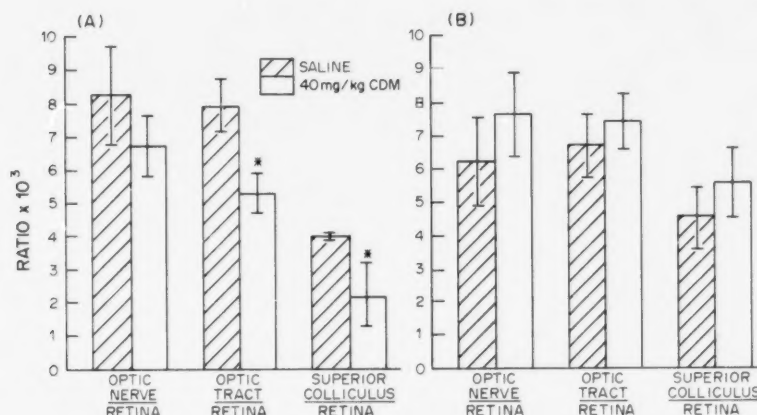


Fig. 5. Degree of rapid axonal transport in the optic system as a function of treatment with chlordimeform (CDM) and body temperature. (A) Chlordimeform-treated rats ($n = 5$) with a body temperature of $35.2 \pm 0.6^\circ\text{C}$ (saline-treated rats: $38.0 \pm 0.5^\circ\text{C}$, $n = 5$) showed a decrease in axonal transport of ^3H -labelled macromolecules to the optic tract and superior colliculus. (B) When the body temperature of the rats treated with chlordimeform ($n = 5$) was raised to $37.9 \pm 0.15^\circ\text{C}$ (saline-treated rats: $38.1 \pm 0.08^\circ\text{C}$, $n = 5$), there were control amounts of ^3H -labelled macromolecules in the optic nerve, optic tract, and superior colliculus of the chlordimeform treated rats. The amount of acid-precipitable radioactivity in each tissue piece is divided by the acid-precipitable radioactivity in the corresponding retina to normalize for variability of isotope injection (* $P < 0.05$, Student's t -test).

dimeform had additional action on pattern-reversal-evoked potentials.

The peak-to-peak amplitude of pattern-reversal-evoked potentials was increased by chlordimeform regardless of body temperature. Although the mean amplitude in the group treated with chlordimeform was increased less at room temperatures of 30°C than at 22°C , the amplitude was still more than twice the control values at 30°C . In addition, the finding of no significant interaction between dose and room-temperature on amplitudes of pattern-reversal-evoked potential confirms that the changes were not indirectly due to hypothermia. It has recently been found that the increase in amplitude of pattern-reversal-evoked potentials is dependent upon the amount of contrast in the visual display (Boyes, Jenkins and Dyer, 1985). If chlordimeform acts on visual contrast-encoding mechanisms, the failure to increase the amplitudes of flash-evoked potentials (Boyes and Dyer, 1985) could result from the strobe-flash stimulus being devoid of contrast.

The rate and amount of rapidly transported proteins is highly temperature-dependent. This has been demonstrated in poikilotherms, such as goldfish (Elam and Agranoff, 1971), garfish (Gross, 1973), swan mollusk (Heslop and Howes, 1972) and frog (Edstrom and Hanson, 1973) and *in vitro* in mammals such as cat (Ochs and Smith, 1971), rabbit (Cosens, Thacker and Brimijoin, 1976), rat and ground squirrel (Bisby and Jones, 1978). The present study demonstrates that mammalian nerves also show decreases in rapid axonal transport *in vivo* with decreases in body temperature. Reduced rapid axonal

transport appears to occur secondarily to the hypothermic effects of treatment with chlordimeform.

The mechanism of action of the reduction of body temperature induced by chlordimeform at normal room temperature is unknown. A variety of actions have been reported for chlordimeform which could interact with thermoregulation, including inhibition of synthesis of prostaglandins (Yim *et al.*, 1978), octopaminergic (Evans and Gee, 1980; Hollingworth and Murdock, 1980; Nathanson and Hunnicutt, 1981), or α -adrenergic agonist/antagonist activity (Robinson and Bittle, 1979), alteration of levels of cerebral monoaminergic neurotransmitters (Johnson and Knowles, 1983), metabolic inhibition (Abo-Khatwa and Hollingworth, 1972) and alteration of concentrations of calcium ion (Zelenski, Robinson and Pento, 1978; Pento, Robinson, Rieger and Horton, 1979). Thus, chlordimeform could reduce body temperature through numerous mechanisms, acting either alone or in combination.

Whatever the mechanism of hypothermia induced by chlordimeform, it is clear that rats treated with chlordimeform at standard room temperatures may become hypothermic. As far as is known, none of the previous studies reporting the consequences of the administration of chlordimeform to whole animals have considered the possible confounding contribution of changes in body temperature. Many of the previous studies, however, have involved lower dose levels than the current study, and changes in body temperature could have been relatively minor. Nevertheless, the results of this study emphasize the importance of changes in body temperature as a

possible factor in studies of intact animals exposed to neuroactive compounds.

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